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Report on Methods of Analysis for Maple Sirup

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The determination of the purity of maple sirup is based upon analytical data for a number of constituents. Important among these are the malic acid values. The current A.O.A.C. method (2) for this constituent is nonspecific; any acid in the sirup that forms a calcium precipitate insoluble in 85% alcohol is measured as malic acid. Last year the Associate Referee and others (3) proposed a method specific for malic acid in maple sirup based upon the method described by Goodban and Stark (1) for the determination of malic acid in plant juices. Since the method gave concordant malic acid values in the Referee's laboratory, it was decided to study it collaboratively. Further, a study of malic acid data for several randomly selected sirups indicated that these values may be nearly constant or at least restricted to a very narrow range, since maple sirup is almost always saturated in respect to malic acid salts. Thus these values might serve as a measure for purity.

Aliquots of a sample of pure maple sirup were submitted to the collaborators together with a copy of the analytical procedure. Each collaborator was requested to make at least 10 separate determinations and to send all results, irrespective of whether they were in close agreement or not, so that the values could be analyzed statistically. The collaborators were also asked to analyze as many other samples of pure sirup as time would permit.

METHOD

Apparatus

(a) *Ion exchange columns.*—Std wall Pyrex glass tubing, 10 mm i.d. × 30 cm long, with 5 cm capillary tip.

(b) *Spectrophotometer.*—Suitable for measuring absorption at 390 mμ, with matched 1 cm cells or matched test tubes.

Reagents

(a) *Ion exchange resins.*—(1) *Cation exchanger.*—Dowex-50 (60–80 mesh). (2) *Anion exchanger.*—Amberlite IRA 400 (60–80 mesh) or Amberlite CG 400, Type I (100–200 mesh).

(b) *Sodium hydroxide soln.*—5%. Dissolve 5 g NaOH, purified grade, in 100 ml H_2O.

(c) *Ammonium carbonate soln.*—0.25*N*. Dissolve 14.26 g $(NH_4)_2CO_3.H_2O$ in enough H_2O to make 1 L.

(d) *Ammonium carbonate soln.*—1.0*N*. Dissolve 57.05 g $(NH_4)_2CO_3.H_2O$ in enough H_2O to make 1 L.

(e) *Sodium carbonate soln.*—1.0*N*. Dissolve 5.3 g Na_2CO_3 in enough H_2O to make 100 ml.

(f) *Hydrochloric acid soln.*—5%. Dil. 12 ml HCl with 88 ml H_2O.

(g) *2,7-Naphthalenediol.*—1 g dissolved in 100 ml H_2SO_4.

(h) *Malic acid std soln.*—Dry Eastman Kodak white label L-malic acid 18 hr at 40°. Dissolve 0.2000 g in 500 ml H_2O. Dil. known vol. of this soln (ca 10 ml) to 100 ml so that final soln gives absorbance, *A*, after reaction with color reagent as in detn, of 0.2–0.8.

Preparation of Ion Exchange Columns

For each column add enough H_2O to 10 ml dry resin to make thin slurry and pour slurry into column contg small plug of glass wool. Let H_2O drain to level of settled resin and wash with 2 ml portions H_2O to condition resins. To cation exchange resin (Dowex-50) add three or four 10 ml portions 5% HCl, letting acid drain to top of resin between each addn. Wash resin free of acid with 10 ml portions H_2O until effluent gives no test for chlorides. (Approx. 4 bed vols of H_2O are required.) Treat anion exchanger (IRA 400) resin with three or four 10 ml portions 5% NaOH, draining liquid to top of resin between addns. Remove excess of alkali with H_2O by washing with 10 ml portions until effluent gives negative alkali pH test with indicator paper. Transform resin into carbonate form by addn of three or four 10 ml portions Na_2CO_3 soln. Wash free of carbonate with 10 ml portions H_2O until effluent is neutral to indicator test paper. Mount conditioned columns vertically with cation resin column directly above anion resin column, connecting tubes with 1-hole rubber stopper mounted in top of anion column. No stopcocks are required; close packing of fine resins prevents liquid from draining below

* Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Table 1. Summary of malic acid values for 1958 collaborative sample of maple sirup

Collaborator	n	Malic Acid, % Max.	Min.	$\bar{x}$	s	v, %
A	10	0.577	0.532	0.549	0.0136	2.48
B	10	0.561	0.515	0.533	0.0163	3.06
C	10	0.581	0.401	0.518	0.0502	9.70
D	10	0.611	0.496	0.553	0.0349	6.31
E	10	0.625	0.505	0.554	0.0439	8.07
F	10	0.555	0.522	0.540	0.0093	1.72
G	10	0.624	0.551	0.579	0.0190	3.28
H	10	0.587	0.554	0.569	0.0114	2.00
I	10	0.586	0.513	0.536	0.0232	4.33
	$\bar{x} = 0.547$		$s_{\bar{x}} = 0.0186$		$v_{\bar{x}}\ \% = 3.40$	

surface of resins. Any portion of resin that becomes dry may be inactivated.

Separation of Malic Acid

Transfer ca 10 ml sirup sample to tared 100 ml vol. flask and weigh to ±0.0002 g. Dil. to mark with H_2O and transfer aliquot contg 6–20 mg malic acid (ca 15 ml) to cation exchange resin and let eluate pass onto anion exchange resin. Wash cation resin (upper column) with three 10 ml portions H_2O, again letting effluent pass directly onto anion resin. Remove upper column and wash anion resin column with three 10 ml portions H_2O to remove sugars and any loosely held acids present that might interfere with test. Elute column with five 10 ml portions 0.25*N* $(NH_4)_2CO_3$ to quantitatively remove all of the glycolic, glyceric, or lactic acids possibly present in original test soln. Elute malic acid from anion resin with five 10 ml portions 1*N* $(NH_4)_2CO_3$; after 45–48 ml eluate collects in 250 ml vol. flask, remove flask and dil. to vol. with H_2O.

Determination

Transfer 1 ml of the malic acid-$(NH_4)_2CO_3$ eluate to 18 × 150 mm culture tube and slowly add 6 ml 96% H_2SO_4 from buret, adding first 2 ml down walls of tube to avoid excessive evolution of CO_2. Add 0.1 ml of the 2,7-naphthalenediol reagent and mix thoroly. Cap tubes with metal culture tube closures and heat in boiling H_2O bath (deepfat fryer is satisfactory) 25 min. to develop color. Cool tubes, and measure absorbances of colored solns within 30 min. in 1 cm absorption cell at 390 mμ against blank of 1 ml H_2O, 6 ml H_2SO_4, and 0.1 ml reagent also heated 25 min. in boiling H_2O bath.

Color developed follows Beer's law in which $a = A/Cb$, where a is absorptivity, C is concn in mg/ml, and b is cell thickness. Absorptivity may vary from day to day because of differences in blank; therefore a must be established daily with duplicate portions of fresh std malic acid soln. Calc. a from absorbance A at 390 mμ of colored soln resulting from reaction of soln of std malic acid and color reagent. Calc. amount of malic acid in sample from: $C = (A/ab) \times$ diln factor. Express value for malic acid in maple sirup in terms of std density (65.5° Brix) sirup.

Results and Discussion

Sixteen chemists who were interested in the problem agreed to participate in the 1958 collaborative study. However, because of difficulty in obtaining the ion exchange resins and other causes, only eight collaborators were able to submit their data in time for this report. The remaining data will be presented in the 1959 report. The results obtained for the 1958 collaborative sample are given in Table 1. The column headings are: n = number of determinations, $\bar{x}$ = mean, s = standard deviation of n determinations, v,% = coefficient of variation,%, $\bar{\bar{x}}$ = average of collaborators means, $s_{\bar{x}}$ = standard deviations of collaborators means, $v_{\bar{x}}$ = coefficient of variation, %, of collaborators means.

The use of this common sample permitted an evaluation of precision of the values obtainable for both inter and intralaboratory analyses. The interlaboratory agreement between the means, $\bar{x}$'s, was very good. The intralaboratory standard deviations show a relatively high precision. However, these data fell into two groups, of which six collaborators' values were 0.023 or less and 3 had s values of more than 0.030. The coefficient of variation, as per cent, (v, %),

Table 2. Summary of malic acid values for a number of maple sirups

Coll.	Sample No.	n	Malic Acid, %[a] Max.	Min.	$\bar{x}$	s	v, %
A	1	10	0.518	0.472	0.496	0.0129	2.60
	2	10	0.446	0.404	0.430	0.0129	3.00
	3	10	0.510	0.470	0.483	0.0136	2.82
	4	8	0.460	0.416	0.433	0.0136	3.14
	5	10	0.445	0.386	0.415	0.0179	4.32
	6	10	0.486	0.410	0.449	0.0246	5.48
	7	10	0.499	0.453	0.482	0.0161	3.34
	8	8	0.429	0.403	0.412	0.0084	2.07
	9	10	0.478	0.453	0.467	0.0077	1.65
C	10	10	0.702	0.503	0.647	0.0570	8.81[b]
	11	10	0.396	0.336	0.367	0.0196	5.34[c]
E	12	10	0.480	0.384	0.443	0.0281	6.35
	13	10	0.468	0.365	0.395	0.0279	7.06
G	14	10	0.680	0.568	0.607	0.0462	7.61
	15	10	0.654	0.626	0.640	0.0089	1.39
	16	10	0.602	0.577	0.590	0.0080	1.36
	17	10	0.504	0.463	0.483	0.0119	2.46

[a] Corrected to 65.5° basis.
[b] Cloudy.
[c] Not of known purity.

which puts the intralaboratory standard deviations on a comparable basis, again shows the close agreement and high precision obtained by six of the collaborators. Although the other three are higher, they too show good precision. Comparing the data as a whole, the average of the collaborators' means, $\bar{x}$, is 0.547 with a standard deviation, $s_{\bar{x}}$, of only 0.0186, having a coefficient of variation per cent of 3.40.

Since none of the collaborators raised questions of major considerations and only two had questions dealing with minor details, it is assumed that the method for malic acid described in this report is amply expressed and readily followed.

The analytical data for malic acid obtained by the collaborators for 17 samples of sirup other than the collaborative one are given in Table 2. The symbols used in the table headings are the same as for Table 1. All of the data were corrected to a 65.5° Brix sirup basis so that the values would be comparable. The highest malic acid value, 0.702%, was obtained for a cloudy sirup, sample 10, indicating that the sirup contained malic acid salts held in suspension, which would account for the high value. Therefore, these data should be excluded. The lowest value obtained, 0.384%, was for Sample 11. The collaborator questioned the purity of this sample. Excluding the data for Samples 10 and 11, the highest and lowest percentages of malic acid found were 0.680% and 0.365%, respectively, with a range of more than 0.3%. These data show that the range of values is greater than desirable if malic acid values were to be used as a criterion of sirup purity. Even so these malic acid values provide a better means of testing sirup purity than any now in use. The standard deviations of these values show the same good precision for the method as obtained for the collaborative sample, Table 1. The coefficient of variation for all 17 samples analyzed in four laboratories has a relatively large range, 1.39 to 7.61, but was less than 3.5 for 10 of these. This indicates that the values obtained by this procedure may be improved by a study of the method of preparing the sample for analysis and other details.

Recommendations

It is recommended*—

(1) That the ion exchange procedure proposed for the determination of malic acid in maple sirup be adopted as first action.

* For report of Subcommittee D and action of the Association, see *This Journal*, 42, 29, 30 (1959).

(2) That the application of the ion exchange procedure described in this report for the determination of malic acid in maple sirup as a measure of the purity of maple sirup be continued.

(3) That malic acid values (Cowles), 29.136, be deleted since the method developed by Cowles in 1908 is obsolete.

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2. Jack Rosenstein, Food and Drug Administration, New York 14, N.Y.

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7. Arthur S. Wendt, Cary Maple Sugar Company, St. Johnsbury, Vt.

8. Philip W. Greer, Food and Drug Administration, Boston 10, Mass.

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